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RECOVERY IN THE DARK OF THE RABBIT'S ELECTRORETINOGRAM

in relation to intensity, duration and colour of light-adaptation

BY

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INTRODUCTION

Ever since the discovery of the electroretinogram (HOLMGREN, 1865), it has been well known that it is easier to obtain big responses, if the eye is dark adapted; yet until recently no one had measured the variation in the size of the b-wave of the electroretinogram (ERG) during the course of dark-adaptation.

CHARPENTIER (1936) performed such experiments on albino rats and found that after intense light-adaptation the amplitude of the b-wave increased in the dark for several hours. His curve of amplitude of the b-wave against time was nearly linear for the first hours. It agreed with TANSLEY's (1931) visual purple regeneration curve for the same animal. Thus, in the 'rod' eye of the rat large electrical responses were found to be associated with high visual purple concentrations.

The dark-adaptation of the ERG in the 'mixed' eye of the frog was followed by WREDE (1937) and RIGGS (1937). In this animal the b-wave is much larger than in the rat, and it was also possible to investigate the early phase of recovery in the dark immediately after the end of light-adaptation. WREDE measured the size of the b-wave elicited by a constant stimulus, and found that after exposure of frog's eye to bright light there was a long delay before the b-wave started to grow. In RIGGS' experiments the intensity of the light stimulus necessary for b-waves of constant size was measured after various times in the dark. The curve obtained showed an initial relatively fast fall followed by a slower decline. RIGGS pointed out that this curve, in terms of the logarithm of intensity against time, is reminiscent of the well known human sensory dark-adaptation curve. KOHLRAUSCH (1922) first demonstrated that dark-adaptation of the human peripheral retina, when expressed in terms of log. intensity of light at threshold, traced a curve with an initial fast drop followed by a slower fall.

GRANIT, HOLMBERG and ZEWI (1938) made parallel measure-

ments of visual purple density and of the size of the b-wave in the frog eye. Their remarkable conclusion was that weak monochromatic lights, which did not bleach any detectable proportion of visual purple, caused a reduction of 30—40 per cent in the size of the b-wave. However, small reductions in the size of the b-wave imply great changes in the sensitivity of the eye. The amplitude of the b-wave is known to be approximately proportional to log. intensity of the stimulating light, and changes in the size of the b-wave of the order mentioned above, must correspond to 100—1000 fold drops in sensitivity (GRANIT, summary, 1952). It has since been confirmed by RUSHTON (1953) and WALD (1954) that rather bright lights have to be used to bleach visual purple effectively. This was also LYTHGOE's opinion (1940). RUSHTON used his opthalmoscopic arrangement for measuring visual purple density *in vivo*, and found that in albino rabbits the normal, maximal density of about 0.2 log. units was maintained in steady illumination of about 100,000 times the human absolute threshold; this intensity is far above the threshold of human photopic vision.

Evidently the b-wave of the ERG can disappear without any measurable break-down of visual purple in the retina, but on the other hand, after effective light-adaptation, great quantities, about one half of the total density, must be regenerated before the b-wave again begins to rise. This was demonstrated in frogs and cats by GRANIT, MUNSTERHJELM and ZEWI (1939). They light-adapted excised frog eyes for one hour to 20,000 lux (m.c.) and afterwards recorded the b-wave evoked by two stimuli of the same wavelength (500 mμ; λ max. of visual purple) one below, the other above the electroretinographic cone threshold of the frog. In response to both these stimuli the faster rise of the b-wave was found to start after about one hour in the dark, which corresponded to a time when about 50 per cent visual purple had been regenerated. ZEWI (1939) in his thesis had elaborated a technique of estimating visual purple concentration and had applied it to similar conditions. Experiments were also performed on cats, decerebrated and light-adapted to 20,000 lux for periods of 30, 10 and 1 min respectively. The longer durations of light-adaptation caused a delayed recovery of the b-wave, and again about 50 per cent of

the total amount of visual purple had to be present before the rising part of the dark-adaptation curve started. The experiments with the cats were carried out to exclude the possibility of pigment migration affecting the results in frogs.

In order to explain these results, GRANIT *et al.* (1939) put forward a hypothesis according to which, in its most general form, there is another stage between the regeneration of visual purple and the recovery of sensitivity as measured by the ERG. According to this view, only a small fraction of the photochemical substance, that on the surface of the rods, takes part in excitation. The great bulk of the visual purple is a physiologically inactive store, from which the active surface is replenished, possibly by diffusion. The intermediate process, (the diffusion to the surface), was assumed not to be able to start before about 50 per cent of the total visual purple concentration has been regenerated.

WALD (1954), has proposed another theory which explains the correlation between the visual purple concentration and the sensitivity of the eye. This 'compartment theory' suggests that the rods are built up of smaller functional elements. These are located in compartments, hypothetically identified with the regular discs of which SCHMIDT (1938) and SJÖSTRAND (1943) found the rods to be composed. Every compartment contains considerable amounts of visual purple (rhodopsin), but if one quantum of light is absorbed by one molecule of the photochemical substance one whole compartment is discharged. It then continues to absorb light but cannot contribute to excitation before all its visual purple has been regenerated. After intense light-adaptation considerable time is needed for regeneration of the visual purple within the structure of the compartments, but before the last molecule is regenerated anyone compartment cannot function. According to WALD, this theory, when expressed mathematically, is consistent with the expectation that the logarithm of visual sensitivity is correlated to the concentration of the photosensitive substance.

GRANIT, THERMAN and WREDE (1938) who studied the dark-adaptation of the frog's ERG, made an interesting discovery. They adjusted monochromatic lights to produce b-waves of constant size, and found, if these lights were used to light-adapt the eye,

they did not cause equal reductions of the b-wave elicited by a test light of wave-length 500 mμ. In general blue and violet lights were less effective in lowering the b-wave, and in some cases adaptation to blue light caused a small increase in the size of the response. They showed that light of wave-length 470 mμ caused the least reduction of the frog's b-wave, provided that the energy-equivalent adapting lights were corrected for visual purple absorption. It seemed probable that some substance (absorbing maximally in the blue region of the spectrum), possibly some of the yellow break-down products of visual purple, enhanced regeneration. The same explanation was suggested by CHASE (1937) and CHASE and SMITH (1939), who found that regeneration of frog's visual purple in solution was faster after exposure to blue or violet light, as compared with the regeneration following an equal exposure to light from the yellow part of the spectrum.

The observations of CHASE and SMITH have been confirmed by HUBBARD and WALD (1952), who explain the effect of blue light on the regeneration in terms of cis-trans isomerization of retinene (vitamin A aldehyde) released during illumination. According to HUBBARD and WALD, the bleaching of rhodopsin yields all-trans retinene, but the retinene combined with opsin (a colourless protein) in rhodopsin, has the cis configuration. For rhodopsin synthesis the all-trans retinene liberated upon bleaching must be converted to the specific cis-isomer called neo-b. Retinene absorbs light maximally in the violet region of the spectrum, and because only light which is absorbed by retinene can isomerize it, light from this region isomerizes more effectively. In the living eye the action of blue and violet light is greatly restricted by the absorption of the lens in this spectral region, but on the other hand, a specific enzyme discovered by HUBBARD (1956), retinene isomerase, catalyses the isomerization process to yield more neo-b retinene when rhodopsin is bleached in the light. Rhodopsin can also be regenerated from the store of neo-b vitamin A in the pigment epithelium and from new vitamin A extracted from the circulation.

As long ago as 1879 KÜHNE had described two types of visual purple synthesis, one from the intermediate break-down products

(anagenesis), and the other, a slower process from the white end products of the bleaching (neogenesis). KÜHNE's anagenesis and neogenesis can be identified with WALD's rhodopsin regeneration from retinene and vitamin A respectively. The difference in the time course of the regeneration from the intermediate and final stages of bleaching, has been proposed as an explanation of the differences in the shape of human sensory dark-adaptation curves as caused by variations in strength and duration of the adapting light (HECHT, HAIG and CHASE, 1937; WALD and CLARK, 1937). In several papers (BLANCHARD, 1918; MÜLLER, 1931; WINSOR and CLARK, 1936; HECHT, HAIG and CHASE, 1937; WALD and CLARK, 1937), it has been shown that the course of dark-adaptation depends both on the duration and intensity of the previous light-adaptation. The second branch of the curve, which indicates the adaptation of the rods (KOHLRAUSH, 1931; HECHT, 1937), is not only displaced in time, beginning later after brighter light-adaptation, but also the shape of the curve is different. The various rod dark-adaptation curves do not trace the same paths. The sensitivity increases much more slowly after intense pre-illumination. According to HECHT *et al.* (1937) the delayed type of rod adaptation, when measured with a violet test light in the normal human eye, first appears after adaptation to intensities about 200 times as great as those producing maximal rod activity in terms of visual acuity, intensity discrimination or perceived flicker fusion frequency.

LYTHGOE (1940) did not agree with the explanation of WALD and CLARK (1937) and HECHT *et al.* (1937) of a different rate of rod adaptation from different stages of visual purple break-down. He pointed out, that visual purple in solution can regenerate from all intermediate stages, but that it is unknown which particular mechanism occurs *in vivo*. LYTHGOE also referred to the discrepancy between the visual purple concentration and the effect evoked in the receptive mechanism, as demonstrated by GRANIT *et al.* (1938) in parallel measurements of the size of the electroretinogram and visual purple density.

There are several reasons for believing that purely nervous mechanisms also play an important part in the adaptive process of the retina. SCHOUTEN (1937) who used a binocular matching method

has shown that the apparent brightness of a test light viewed foveally, can be depressed by a separate illumination of the retinal periphery. The reduction of brightness occured in 0.1 sec and reached a steady level, which then was maintained. This process, which he called α-adaptation, cannot be explained photochemically, it is both too rapid and is also effective at a distance, in fact, from as far away as from the blind extreme temporal part of the retina.

ADRIAN and MATTHEWS (1927) recorded both the electroretinogram and the optic nerve response in the eye of the eel, and found that the latent period of the response was shorter if the intensity of the stimulus or the area stimulated was increased. The area within which this interaction could be demonstrated was found to be 1 mm in diameter.

The overlapping retinal areas which functionally are connected to single ganglion cells, called receptive fields, have since been measured and have a maximal diameter of about 1 mm in the frog (HARTLINE, 1940), and a diameter of 1 to 2 mm in the cat (KUFFLER, 1953). KUFFLER also found that the receptive fields shrink in bright light and expand in the dark. BARLOW (1953) has demonstrated in the frog that the discharge from an isolated retinal ganglion cell to a patch of light smaller than its receptive field can be inhibited when a small area outside its receptive field is simultaneously illuminated.

As might be expected experiments on human perception reflect the complicated functional organization of the retina. The sensory dark-adaptation curves show typical differences depending on the size and the time of exposure of the test-field (CRAIK and VERNON, 1941; ARDEN and WEALE, 1954; RUSHTON and COHEN, 1954). For larger test-fields the dark-adaptation is both faster and covers a greater range in log. units. When measured with a small test-field (which greatly diminishes summation in the rods) the foveal and extra-foveal thresholds of dark-adapted eye are nearly the same (BAUMGARDT, 1949; ARDEN and WEALE, 1954; WEALE, 1958).

At the moment it is not exactly known to what extent the increasing concentration of the visual purple contributes to the

rising sensitivity of the eye during dark-adaptation, and to what degree the size of the receptive fields determines the threshold of the retina. However, in the visual process the first step leading to the discharge of nerve impulses is the absorption of light quanta by the photopigment molecules in the receptors. It is clear that, if the photochemical substance has been totally bleached, it must be regenerated before the visual process, based on visual purple, can function. The absorption curve of visual purple in solution agrees well with the human scotopic visibility curve, as first shown in 1894 by KÖNIG (collected papers 1903) and since repeatedly confirmed. The same curve can be reproduced by measuring the size of the electroretinogram to monochromatic low intensity stimuli, shown for example in the frog eye by GRANIT and MUNSTERHJELM (1937). The b-wave is the only component of the electroretinogram known to take part in excitation (GRANIT, 1933), and its amplitude is known to be proportional to the logarithm of the stimulus intensity. Therefore, it can be expected that recovery in the dark of the b-wave intimately reflects the increase of the sensitivity of the eye. This has been confirmed by KARPE and TANSLEY (1948), who showed that in the human eye, after intense light-adaptation, the size of the b-wave of the electroretinogram fits the simultaneously measured subjective dark-adaptation curve if both curves are made to coincide at the starting point of the second branch of the sensory curve. On the other hand it must be concluded, that the size of the electrical response does not measure the concentration of visual purple in retina. If the eye has been illuminated with a weak light, the full amount may still be present, despite a small ERG; alternatively, after intense light-adaptation, a b-wave of the same size can be obtained in response to the same test light when only about one half of the final concentration of the visual purple has been regenerated.

THE PROBLEM

The present work deals with the scotopic b-wave of the electroretinogram in anaesthetized rabbits during dark-adaptation. The duration and intensity of the white adapting light have been varied over a wide range. The amplitude of the b-wave, elicited by a constant monochromatic test light at 500 mμ, has been measured over long periods of time in the dark. The test light was chosen to give about 90 per cent of the maximal b-wave in fully dark-adapted eye. For every experimental animal the light-adapting intensities have been compared with the intensity of the constant green test light, using the 50 per cent maximal b-wave as the common index. The electroretinographic cone threshold has been determined by using the flicker method.

It has been found important in this work to have spectral stimuli precisely defined by the criterion of visual purple equivalence in all wave-lengths. For this reason a great deal of attention was initially given to such measurements in terms of the b-wave.

The spectral sensitivity of the rabbit's retina has been measured by finding the light intensity which gave a b-wave of constant size (50 per cent of maximum) in the fully dark-adapted eye. The recovery of the b-wave has been studied after adaptation of the eye to scotopically equivalent blue and orange lights, which at one intensity level were clearly below the electroretinographic cone threshold, at the other just above it.

The following problems have been raised:

1. How long after intense light-adaptation does the delay last before the scotopic b-wave reappears, and how long is the maximal recovery time of the b-wave in the dark?

2. What is the lowest intensity which definitely causes slow recovery of the scotopic b-wave, as compared both with that necessary to obtain maximal b-waves in a fully dark-adapted eye, as well as with the illumination at the break in the ERG flicker fusion curve?

3. Is there any measurable difference in the recovery time of the scotopic b-wave after adaptation to blue light as compared with scotopically equivalent illumination with orange light?

The results show that the definitely delayed recovery of the scotopic b-wave is present, only if the previous light-adaptation has been strong enough to stimulate the cones effectively. Comparisons have been made between strength and duration of preceding light-adaptation. Within limits, these can replace each other in producing slow dark-adaptation in terms of size of b-wave. Various controls have been used in order to establish the level at which signs of cone activation appear. It is suggested that activated cones suppress the function of rods. More evidence for this view was found when the dark-adaptation of totally colour blind human beings was studied electroretinographically (ELENIUS and HECK, 1957, 1958). In these eyes the b-wave started to rise immediately after previous light-adaptation of a strength which in the normal control eyes caused a period of delay of several minutes.

METHODS

Stimulation. In most experiments the eye was stimulated with single flashes of light and adapted with a conditioning light. In some experiments flickering light was used for stimulation. The single flashes were obtained with a camera shutter in the light beam, whereas the flickering light was obtained by a rotating sectored disc with a Velodyne motor-generator as described by ENROTH (1952).

The optical system used was partly based on a Hilger-Tutton monochromator described by GRANIT and MUNSTERHJELM (1939) and partly on interference filters. Two Philips tungsten lamps with a vertical ribbon filament (15 A, 6 V) were used for the two light beams. The lamps were connected in series and supplied with current from a stabilized power source. One lamp was run at 2800 K° and focused on the collimator slit of the Hilger-Tutton monochromator. The other lamp, run at 2856 K°, was used with Balzer's double-interference filters placed in a parallel section of the beam. The interference filters (2 × 2 cm) were mounted in a revolving disc, in which were cut twelve round holes, 16 mm in diameter. The transmission maxima of the filters used were at 400, 420, 436, 463, 480, 498, 520, 546, 580, 598 and 620 mμ respectively. One hole in the disc was left open for white light. The transmission curves of the filters λ max. 420 and 580 mμ are given in Fig. 1. The filter properties were measured with a Unicam spectrophotometer (No. SP 600). The maximal transmission of the Balzer interference filters used varied between 42.5 per cent (λ max. 400 and 598 mμ) and 27.5 per cent (λ max. 546 mμ). The band widths of the filters (1/100 of maximal transmission) varied between 42 mμ (λ max. 520 and 598 mμ) and 79 mμ (λ max. 620 mμ). In spite of the relatively high maximal transmission, the energy transmitted by the blue and violet filters is low at 2800 K°. For this reason, in some experiments where intense blue illumina-

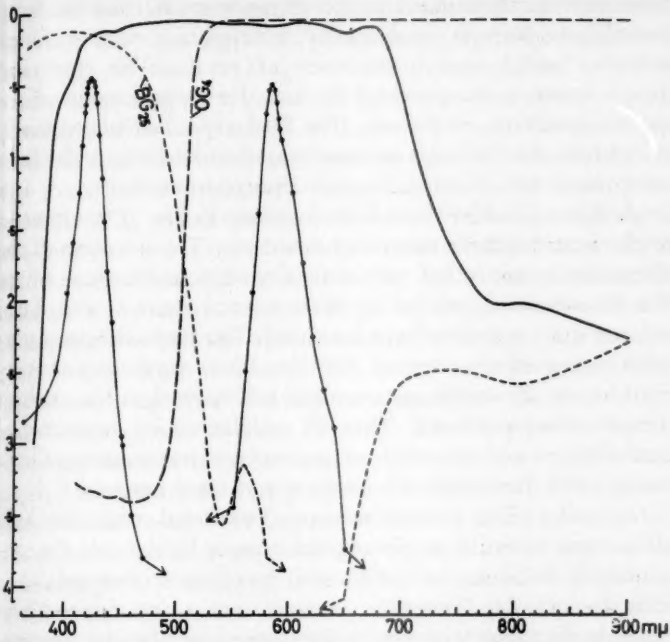

Fig. 1. Transmission curves of some filters used in this work. *Ordinate:* optical density as measured with Unicam SP 600 spectrophotometer. *Abscissa:* wavelength in millimicrons. *Dotted lines:* Balzer interference filters λ max. 420 and 580 mμ. *Broken lines:* Schott Jena filters OG4 and BG25. *Solid line:* Balzer heat filter 'Calflex'. Beginning from arrows (at the base of the curves) the density exceeds 4 log. (transmission less than 1/10000).

tion was required, the broad band Schott Jena filters BG 25 and OG 4 were used. These filters divide the visible spectrum in two parts at about 500 mμ and transmit the blue or the orange side respectively (Fig. 1, broken lines).

The energy content through the spectrum at constant colour temperature was carefully examined for both lamps and monochromator sets by means of a Hilger vacuum thermoelement connected to a sensitive Siemens Super-galvanometer and to a magnifying optical system for reading the deflexion of the galvano-

meter mirror. The equivalent energy spectrum was derived from several galvanometer readings by interpolating to a constant deflexion using constant exposures of 10 sec. The equivalent energy spectrum has provided the basis for measurements of the spectral sensitivity of the eye. The final experimental sensitivity curves have been plotted on equal quantum intensity to facilitate comparison with the visual purple absorption curve.

A Balzer ('Calflex') heat filter (solid line in Fig. 1) was inserted in the beam of both monochromator sets. The intensity of the illumination was varied with neutral wedges and neutral filters. The Bausch and Lomb steel powder neutral filters in sets of 0.3, 0.6, 0.9 and 1.2 density have been used. The filters deviate somewhat from perfect neutrality. For these filters the density at every tenth mμ in the visible spectrum and for the wedges the gradient density were measured. Thus all calculations of experimental results were based on actual measurements of transmission of both wedges and filters with the lamps run at rated currents.

Recording. The electroretinogram associated with the light stimuli was recorded on photographic paper by the aid of a conventional RC-coupled differential amplifier connected to a cathode-ray tube. The time constant was 1 sec. Silver-silver-chloride electrodes with cotton wicks were used for the measurements. The recording electrode was placed on the cornea while the reference electrode was placed in an operation wound on top of the head. It was checked that the electrodes were sufficiently free from photoelectric effects so as not to distort the measurements.

Part of the light beam used for stimulation was applied to a gas filled photocell. The response of this cell was partly used for marking the time of stimulation on the records, partly to synchronize the sweep generator for the CRT with the stimulus.

Preparation and procedure. For spectral sensitivity measurements both pigmented and albino rabbits have been used. All other experiments were made on pigmented animals only. The rabbits were anaesthetized with 20 per cent urethane in Ringer's solution. About one third of the total dose of 1.5 g/kg body weight was given intravenously, the rest injected subcutaneously. The trachea and femoral vein were cannulated. Flaxedil in Ringer's solution was

given to stop eye movements. This made artificial respiration necessary. About 1 mg of the substance per kg body weight and hour was required to keep the animal immobilized. Rabbits smaller than 2 kg were not used. The pupils were dilatated with homatropine and Veritol (Knoll) drops, the eye-lids were cut vertically and fixed to the surrounding skin with four sutures so that the whole eye was well exposed down to the fornix conjunctivae. The animal was mounted in a head holder and placed in a dark box and warmed with hot water bottles. The two light beams were directed by prisms to the eye examined. A green light of wave-length 500 mμ from the Hilger-Tutton monochromator was adjusted to fall straight on the eye in a parallel beam which filled the pupil. This beam made an angle of 15 degrees with the other. The latter was focused on the cornea with a 10 D convex lens of 4 cm diameter. This lens was filled by the beam and the area of the focus was smaller than the well dilatated pupil. All experiments were performed on intact eyes. The retinal area of about 7—8 mm in diameter which was directly illuminated was adjusted to fall on an area below the optic nerve head which according to KÜHNE (1879) is in the rabbit rich in visual purple. In the retina the maximal intensity of white light obtained with the apparatus used, was calculated to be about 8000 lux, as measured with a photoelectric luxmeter at the level of the focusing lens.

After the operation the animal was left in the dark box for at least three hours before the experiment was begun. During this period the state of the retina was checked from time to time by recording an ERG with a single short flash of green light from the Hilger-Tutton monochromator. The intensity of the test light was chosen, to give on an average, 90 per cent of the maximal response (see Fig. 10). Light flashes of 0.3 to 0.2 sec duration were used (Ibsor shutters in both beams). Artificial respiration and Flaxedil injections were started after about two hours of dark-adaptation. In most animals b-waves of 0.3 to 0.5 mV were obtained after three hours in the dark. No experiments were done on rabbits which had b-waves smaller than 0.3 mV after three hours dark-adaptation. In some ten per cent of all animals examined, the ERG-response in full dark-adaptation was surprisingly

small, a fact which is assumed to be due to individual variations in the development of the retina in these animals. On the other hand rabbits which had large b-waves after three hours in darkness could be light-adapted repeatedly and the subsequent dark-adaptation measured for several hours. With careful treatment of the animal the same maximal amplitude of the b-wave could be obtained several times during an experiment lasting up to ten hours.

RESULTS

Spectral sensitivity of the dark-adapted rabbit's retina

Both pigmented and albino rabbits have been examined. The animals were dark-adapted for three hours before beginning the experiments. The energies necessary for b-waves of constant size (50 per cent of the maximum) were measured. These were elicited by flashes of 0.2 sec duration repeated at intervals of 30 sec. For every wave-length three b-waves were photographed at four levels of stimulus intensity. Wave-length 500 mμ was used as running control and repeated in every third series of measurements. In the subsequent calculations of sensitivity curves, the energy of the control at 500 mμ was always scaled as 100 per cent. The final results, as stated, are in terms of quantum sensitivity.

For three pigmented and three albino rabbits the Hilger-Tutton monochromator was used. The averages of the results given in Fig. 2 (crosses and open circles) agree with the results of WIRTH (1953) and DODT and WALTHER (1958*a*) in showing that for the albino retina there is a narrower sensitivity curve than the absorption spectrum of visual purple and there is an additional hump in the red part of the spectrum. In Fig. 2 the difference in sensitivity between the pigmented and albino retinae is over 0.5 log. unit at 600 mμ and over 1 log. unit at 620 mμ. The sensitivities of the pigmented eyes nearly agree with WALD's (1949) visual purple absorption curve of the frog. This result was obtained with both the Hilger-Tutton monochromator (crosses) and the Balzer interference filters (filled circles). Both crosses and filled circles represent averages of three experiments. Regular deviations from visual purple absorption curve were found only at 400 and 420 mμ (see also Fig. 14). These deviations are possibly caused by absorption and fluorescense in the eye media (*cf.* DODT and WALTHER, 1958*b*,*c*). The measurements of DODT and WALTHER

Fig. 2. Electroretinographic measurements of spectral sensitivity of dark-adapted rabbit (Urethane, Flaxedil). *Open circles* are averages from experiments on three albino rabbits and *crosses* from three pigmented rabbits (Hilger-Tutton monochromator stimulation). *Filled circles* are averages from three experiments on pigmented rabbits stimulated with Balzer interference filters. Solid line is Wald's (1949) absorption spectrum of frog's rhodopsin.

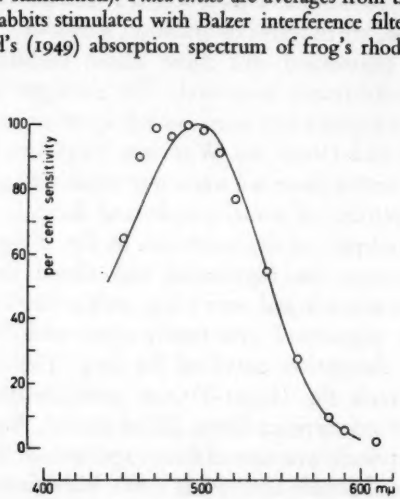

Fig. 3. *Circles:* Averages from experiments on two pigmented rabbits both showing especially high sensitivity in blue spectral region. *Solid line:* Dartnall's (1953) absorption spectrum of visual pigment 497.

(1958*a*) in aphakic rabbit eyes agree both with the α and β-band of visual purple absorption curve.

In two of the six pigmented eyes examined a definite rise of sensitivity was found at 460—470 mμ (Fig. 3). DODT and ELENIUS (1956) who lead off with platinum microelectrodes from isolated retinal ganglion cells and used slow intermittent stimulation found similar deviations from the visual purple absorption curve. They also found that light-adaptation enhanced the blue and violet sensitivity of the rabbit's retina.

In all the nine eyes examined (albinos included) the maximal scotopic sensitivity was found at 490, 498 or 500 mμ. The deviations in the blue at 460—470 mμ from the visual purple absorption curve were small in the averages and only just recognizable in the logarithmic plot used to emphasize the hump in the red part of the spectrum. According to DODT and WALTHER (1958*a*) the narrow sensitivity curve and the great red sensitivity of albino retina lacking pigment epithelium is caused by reflexion of incident light from choroidal blood with consequent double absorption of the reflected wave-lengths in the visual pigment.

Recovery in the dark of the scotopic b-wave after light-adaptation with intense white light

After intense light-adaptation recovery of the b-wave takes place in two phases (see Introduction), the initial slow phase and the later faster phase. Both have been followed in this work. Some experience of the late recovery had already been obtained in the course of the measurements of the spectral sensitivity described above. It was noted that the b-wave of the running control (500 mμ) continued to increase beyond the period of three hours of preliminary dark-adaptation which it was originally hoped would be sufficient for the preparation to stabilize.

In this series of experiments the test response was elicited with 0.3 sec flashes of parallel light of wave-length 500 mμ from the Hilger-Tutton monochromator. This provoked a 90 per cent maximal b-wave in the fully dark-adapted state. White light (of 2856 K°) was used for light-adaptation and focused on the cornea. As with

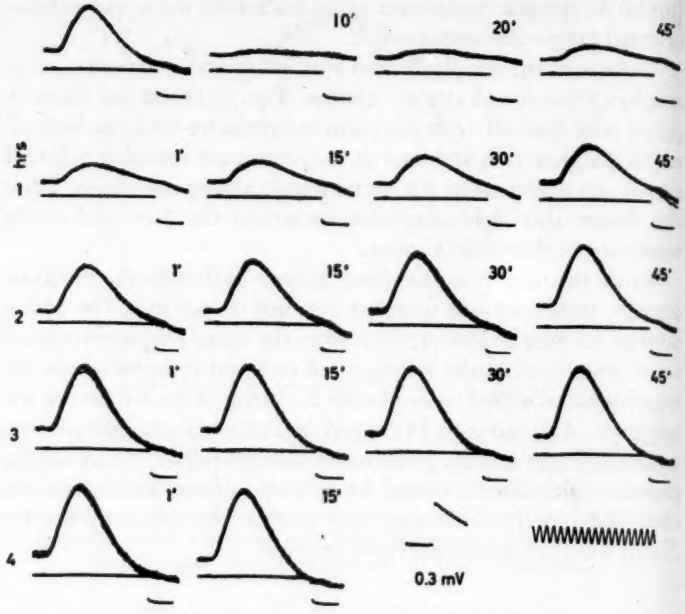

Fig. 4. Rabbit electroretinograms recorded during dark-adaptation following light-adaptation to intense white light (8000 lux, 30 min). The constant test light used gives about 90 per cent maximal b-waves in full dark-adaptation. Five b-waves superimposed in every record. The record in upper left corner is a control (after three hours dark-adaptation) before light-adaptation. Time in the dark after light-adaptation is given in hours (to left) and the minutes over the hours are marked in the upper right corner of the respective records. Photo cell signal lightperiod upwards. Calibration 0.3 millivolts. Timemark 50 c/s.

the present apparatus the retinal illumination could not be increased beyond 8000 lux, long durations of illumination, up to 30 min in one series of experiments, were used to light-adapt the eye as effectively as possible. In every experiment the animal was first dark-adapted for three hours. At the end of this period the size of the b-wave produced by the test light was checked. In every record five sweeps were superposed at 15 sec intervals, except in the experiments in which three and one minutes of

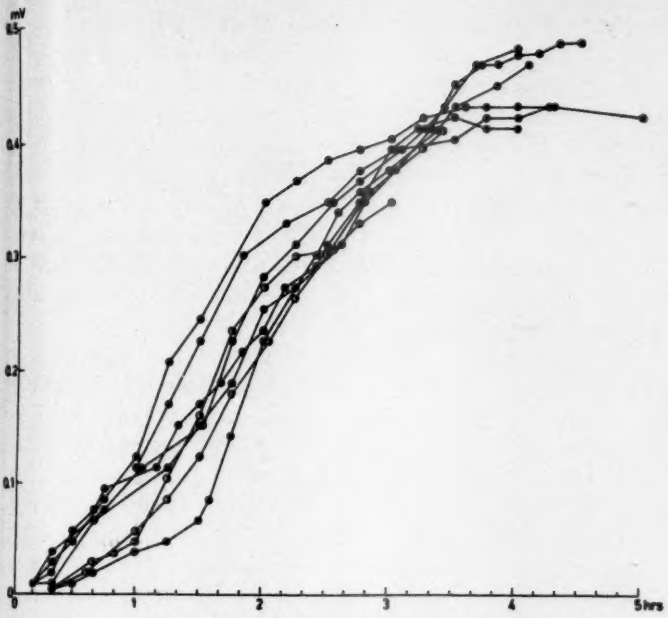

Fig. 5. Dark-adaptation curves of eight pigmented rabbits after light-adaptation to intense white light (8000 lux, 30 min). *Ordinate:* amplitude of test b-wave in millivolts. *Abscissa:* time in hours after the end of light-adaptation.

light-adaptation were used. In these the initial dark-adaptation was so fast that it had to be measured with single b-waves.

The records in Fig. 4 are all from one experiment in which the eye was light-adapted for 30 min to 8000 lux. The control b-wave after the preliminary three hours of dark-adaptation is given in the upper left corner. The other records are taken after light-adaptation, and show clearly how slowly the b-waves grow during the first 20 minutes in the dark. Faster recovery begins at about 45 min and then continues for about two hours. From the third to fourth hour in the dark the b-wave continuously but slowly increases in amplitude.

Recovery curves from eight experiments including the one described above are given in Fig. 5. The records from Fig. 4

represent one of the fastest dark-adaptations in this series (the only curve ending at five hours in Fig. 5). In these curves the period of delay before the b-wave reappears after constant light-adaptation of constant duration varies from 10 to 20 min, and the faster phase in the rise of the electrical response starts on an average after about one hour in the dark. Two rabbits (the half filled circles) were only followed for three hours and 15 min, in the other six cases the recovery was measured at least for four hours. In three cases the curves flatten out after about four hours in the dark, in three other cases the increase of the b-wave still continues after four hours and shows no definite plateau.

In Fig. 6 average recovery curves from several experiments are given in per cent of maximal b-wave amplitude. The curves are from three series of experiments in which the duration of light-adaptation was varied. Open circles represent the average values for six animals which were light-adapted for 30 min (filled circles in Fig. 5), half-filled circles five cases light-adapted for 10 min, and the filled circles five cases light-adapted for 3 min only. The crosses are *in vivo* measurements of albino rabbit's visual purple densities during regeneration in darkness (RUSHTON, CAMPBELL, HAGINS and BRINDLEY, 1955) which have been replotted in per cent of maximal density attained in full dark-adaptation. After intense light-adaptation of long duration, a delay of about 10 min in the recovery of the scotopic b-wave (open circles) corresponds to about 25 per cent of visual purple regenerated in the curve of RUSHTON *et al.* At one hour in the dark (open circles) the b-wave is still only about 15 per cent of the maximum in spite of the fact that nearly all (more than 95 per cent of the maximum) of the visual purple has been regenerated.

According to RUSHTON *et al.* (1955) the total regeneration time of visual purple in the albino rabbit is about 80 min. Of the electroretinographic dark-adaptation curves in Fig. 6 only one has reached its maximum in this time. This is the curve representing recovery after light-adaptation for 3 min. After 10 min of light-adaptation (half filled circles) the average curve approaches its maximum about one hour after full visual purple regeneration; after 30 min of light-adaptation (open circles) not until two and

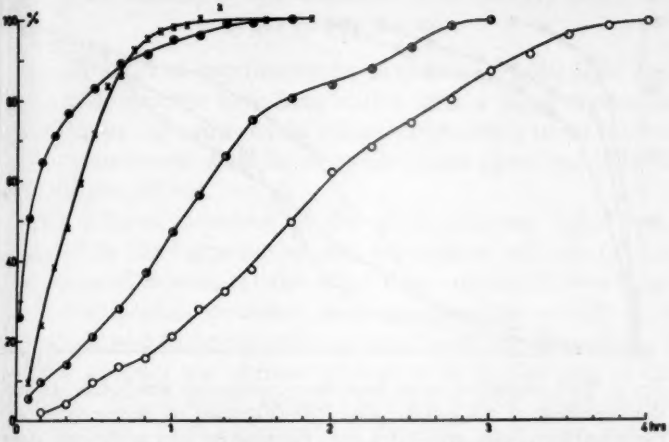

Fig. 6. Average dark-adaptation curves in per cent of maximal b-wave amplitude. Preceding light-adaptation to intense white light (8000 lux): *open circles*, for 30 min (six cases); *half filled circles*, 10 min (five cases); *filled circles*, 3 min (five cases). *Crosses* are *in vivo* measurements of albino rabbit's visual purple densities during regeneration in darkness (Rushton et al. 1955).

a half hours later. If the only basis of comparison is that of equal b-waves, it is clear that anyone visual purple concentration would correspond to widely different times of recovery of b-waves *i.e.* a number of lines drawn parallel to the abscissa will cut across points on the curves differing in time from one another by up to several hours.

The rabbits used in the experiments of RUSHTON *et al.* (1955) were decerebrated but some urethane was also given for immobilization. In the present experiments urethane anaesthesia and Flaxedil was used and as it is likely that the condition of decerebrate rabbits would never have stayed constant for the times needed. Some decerebrate cats were tried. Their b-waves, after intense light-adaptation did not regain the original amplitude (determined after three hours in the dark), as those of the rabbits' did with perfect regularity, despite anaesthesia, Flaxedil and artificial respiration.

The extremely slow recovery in the dark of the rabbit's b-wave after intense light-adaptation might be due to the anaesthesia slowly

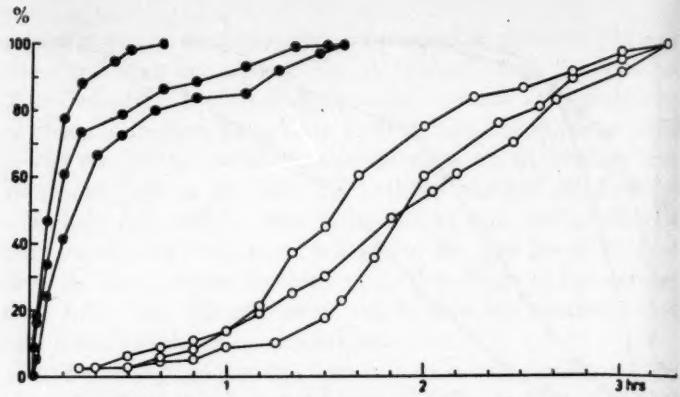

Fig. 7. Dark-adaptation curves from three experiments which show an initial slow recovery of test b-wave after light-adaptation to intense white light (8000 lux) for 30 min, *open circles*, and a fast recovery later in the same experiments after a second light-adaptation to the same strength of light but for 1 min only, *filled circles*. The size of b-wave at 3 hours 15 min in darkness after first light-adaptation represents 100 per cent of amplitude.

wearing off during the experiments, which lasted many hours. This was checked with two rabbits in which, beginning at about four hours in the dark, a plateau was found in the recovery curves of the b-wave. In these cases 3 ml of 20 per cent urethane solution was given intravenously without any change of plateau level.

It was also important to know if an intensely light-adapted eye, that had showed a greatly delayed initial recovery of the b-wave, would be capable after this of producing fast recovery to a brief light-adaptation of the same strength. In three rabbits with a greatly delayed initial recovery of the b-wave (Fig. 5, the half filled circles and the curve showing the slowest dark-adaptation of them all) the eyes were light-adapted to maximal illumination a second time, but only for one minute. In all three cases a rapid second recovery of the b-wave was found. In Fig. 7 the three curves from Fig. 5 are replotted in per cent of b-wave amplitude (open circles). The amplitude at three hours 15 min in the dark represents 100 per cent of b-wave amplitude. The filled circles show the fast dark-adaptation after repeated light-adaptation of one minute duration, plotted in per cent of the same maximum.

Effect of the intensity of light-adaptation on the recovery in the dark of the scotopic b-wave

In this series of experiments the intensities of white light used for light-adaptation have been varied over a range somewhat exceeding six log. units and the subsequent recovery of the b-wave has been measured with the monochromatic green test light of wave-length 500 mμ.

The relative intensities of the white adapting lights were measured in the beginning of each experiment with the (50 per cent maximal) b-wave as index rather than a photocell. Four hours of dark-adaptation preceded these measurements which were carried out with flashes 0.2 sec in duration. In the scale thus obtained the zero intensity was ultimately taken to be the intensity of the test light. This, as stated, caused a scotopic b-wave about 90 per cent maximal. Each experiment is therefore, as it were, individually scaled to the functional properties of the retina used in as much as they are expressed by the scotopic b-wave.

The data of this section are results from individual experiments, in which the eye was light-adapted and dark-adapted several times in succession, but the sensitivity of the eye remained constant to within 10 per cent throughout, as judged by size of b-wave at the end of each dark-adaptation. The results which stood up to this criterion are from eight experiments on pigmented rabbits. Two durations of light-adaptation have been used, 10 min in one group of experiments and 3 min in the other. In both groups one half of the experiments were begun with light-adaptation to maximal intensity (after the preliminary dark-adaptation) and in the other half with the weakest illumination in the series of light-adaptations.

The curves in Fig. 8 are averages of results from four experiments in which the light-adaptation lasted 10 min. Six different intensities of light adaptation were used. Following symbols are used for the different relative intensities, using the scale defined above: + 2.86, crosses; + 1.77, open circles; + 0.78, filled circles; − 0.27, open triangles; − 1.19, filled triangles; and − 2.09, half filled circles. The intensities, too, are averages from the four experiments.

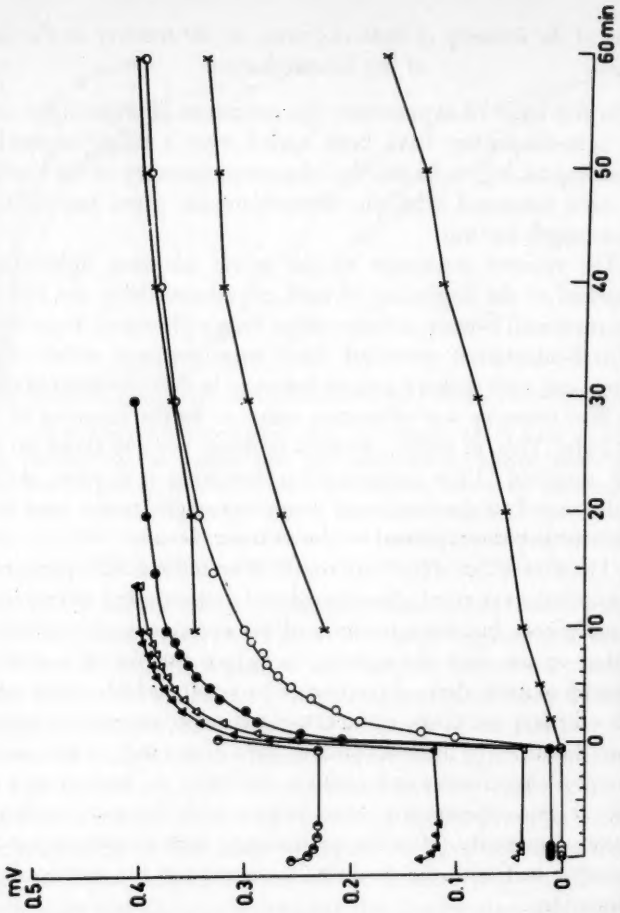

Fig. 8. Average light and dark-adaptation curves from four experiments on pigmented rabbits. Preceding light-adaptation to six different intensities of white light for 10 min. The symbols used and the respective log. relative intensities of light-adaptation are: *crosses*, + 2.86; *open circles*, + 1.77; *filled circles*, + 0.78; *open triangles*, —0.27; *filled triangles*, — 1.19; *half filled circles*, — 2.09. The intensities are expressed as multiples of the effect of the constant test light (zero intensity). *Ordinate:* size of the test b-wave in millivolts. *Abscissa:* time in minutes, zero time in the end of light-adaptation. The slow dark-adaptation curve marked with crosses is at 60 and 120 min shifted back to zero time. First measurement during light-adaptation is at 15 sec. First measurement in the dark is 30 sec after the end of light-adaptation.

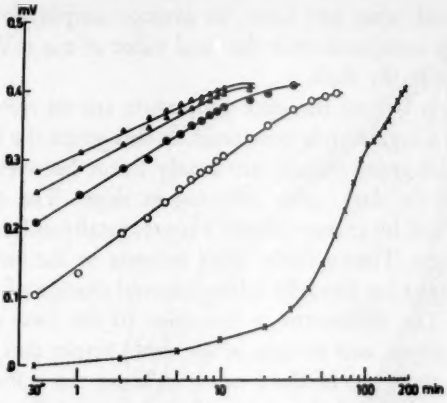

Fig. 9. Dark-adaptation curves from Fig. 8 replotted using logarithmic time scale.

During light-adaptation the amplitude of the b-wave is considerably reduced even by quite weak illuminations. This reduction, to whatever level it may go, is almost instantaneous. There are only small changes in the amplitude of the b-wave after the first measurement made at 15 sec. In the fully dark-adapted eye an illumination (open triangles) of nearly the same intensity as that of the green test light reduces the response to about 10 per cent of the original maximal amplitude (see also below, Fig. 10).

After light-adaptations which do not abolish the b-wave (intensities between + 0.78, filled circles and — 2.09, half filled circles) its recovery in the dark is very fast. The half time of recovery is about 30 sec. Slightly slower recovery (half time about 2 min) is found after light-adaptation to log. relative intensity + 1.77 (open circles) which just abolishes the response to the test light. In this curve after 10 min in the dark, the b-wave is about 80 per cent maximal as compared with some 90 per cent after light-adaptation with the ten times weaker illumination log. relative intensity + 0.78 (filled circles). A quite different time course of recovery is found if the adapting light is increased to log. relative intensity + 2.86 (crosses). After this less than ten per cent of the b-wave has reappeared during the first 10 min in

the dark and, after one hour, its average amplitude is still only 0.16 mV as compared with the final value of 0.4 mV found after three hours in the dark.

In Fig. 9 five of the dark-adaptation curves from Fig. 8 are plotted on a logarithmic time scale. In this graph the fast recovery curves (four upper curves) are nearly linear between 30 sec and 10 min in the dark, only differing in slope. The slowly rising curve marked by crosses follows a course totally different from that of the others. Thus a fairly small increase in the strength of the adapting light has brought a fundamental change of behaviour in recovery. The differences in the slope of the four upper curves (between 30 sec and 10 min in the dark) imply that within these limits the recovery of the b-wave is faster the more intense the light-adaptation and the greater the reduction during illumination. The upper limit is + 1.77 on our scale. If one goes one log. unit above this intensity the recovery is considerably slower.

Results from individual experiments are illustrated in Figs 10 and 11. The four experiments already described (averages obtained after 10 min of light-adaptation in Figs. 8 and 9) are here marked with triangles. Another similar group of experiments in which the duration of light-adaptation was 3 min is marked with circles.

In Fig. 10 the curves marked by crosses represent the size of the b-wave evoked by single flashes of intensities equal to those of the various adapting lights. The measurements are made in three pigmented rabbits, dark-adapted over four hours. These curves, which coincide at 100 per cent amplitude, show that the green test light (= zero abscissa) produces nearly maximal b-waves in the fully dark-adapted eye. The curves are sigmoid in shape, but practically linear within a considerable range.

The main purpose of Fig. 10, however, is to illustrate some aspects of adaptation and early recovery in the dark. Thus open triangles and circles show the reduction of the b-wave in per cent of amplitude, at the stable level reached during the light-adaptations as a function of log. adapting intensity. These curves cover approximately the same range in log. units as those of b-wave amplitude against log. stimulus intensity (crosses). They show that light intense enough to produce maximal b-waves (single flashes)

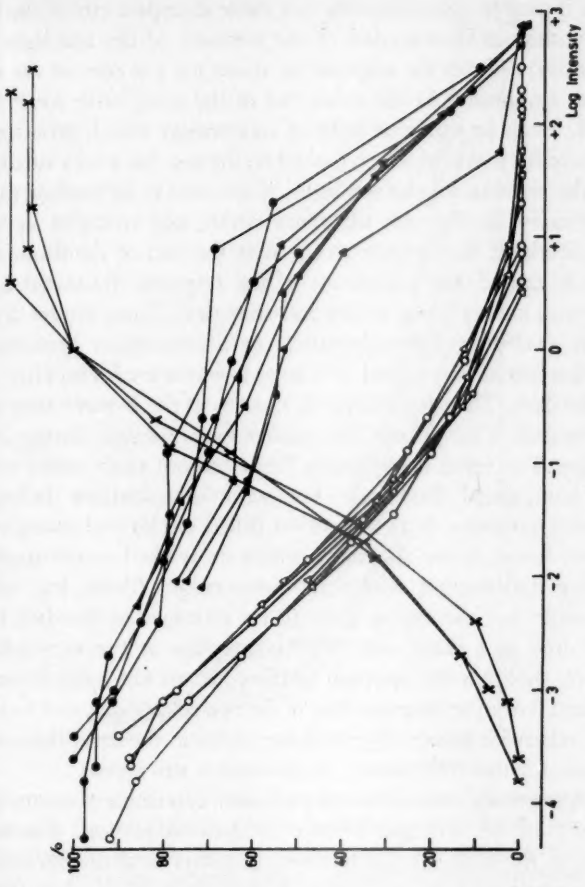

Fig. 10. Individual measurements from eight experiments of reduction of b-wave during light-adaptation, *open circles and triangles*, and of size of b-wave after 30 sec recovery in the dark, *filled circles and triangles*, plotted in per cent of the b-wave evoked by the constant test light (zero intensity) in the dark-adapted eye, as ordinate against log. intensity of light adaptation as abscissa. Two durations of light-adaptation: *circles*, 3 min and *triangles*, 10 min. The *crosses* (three cases) illustrate the amplitude of the b-wave against log. intensity of stimulating light (0.3 sec flashes) in dark-adapted eye.

will, if used to light-adapt the eye, cause disappearance of the ERG. A continuous illumination of the intensity of the test light (zero intensity) reduces the response to about ten per cent of the maximum amplitude. At the other end of the scale, with weak intensities, it can be seen that light of an intensity which produces just measurable b-waves, when flashed on the eye, has a very small effect on the response to the test light, if it is used to light-adapt the eye.

Finally, in Fig. 10, the filled circles and triangles show the amplitude of the b-wave 30 sec after the end of the illumination which caused the reductions of the response illustrated by the previous curves (open circles and triangles). These curves demonstrate that after light-adaptation to illuminations between log. relative intensity — 4 and + 1 large b-waves are found after 30 sec in the dark. The total change in the size of the b-wave after 30 sec in the dark is the greater, the smaller the amplitude during illumination. This result is implied in Fig. 8 but not easily seen owing to the compressed time scale. For weak illuminations (below log. relative intensity + 1) the curves (filled circles and triangles) are nearly linear, as are also those which show the b-wave amplitude during illumination within the same range. Above log. relative intensity + 1 the curves after 30 sec recovery in the dark inflect and drop at a faster rate. This takes place at the very intensity which abolishes the response to the constant test light. It can also be seen from the diagram that if the eye is light-adapted to nearly log. relative intensity +3, after the 30 sec in the dark (the moment chosen for this comparison) the b-wave is still absent.

Apparently two different processes determine the rate of recovery of the scotopic b-wave in dark-adaptation. The second process, the slow delayed recovery can only be demonstrated after light-adaptation to intensities higher than about log. relative intensity + 1. It is important that the inflection of the 30 sec recovery curves coincides with the intensity which produces maximal b-waves in the fully dark-adapted eye (curves marked by crosses) and that this is the intensity which just is high enough to abolish the response to the constant test light. The intensity scale of the abscissa was based on the response to the test light in full dark-adaptation in order to make these comparisons possible.

Fig. 11. Later time course of dark-adaptation in the same eight experiments as illustrated in Fig. 10. *Ordinate:* time (in minutes) necessary for the b-wave to increase from 60 per cent level to 90 per cent of maximum. *Abscissa:* log. intensity of preceding light-adaptation. Duration of light-adaptation: *filled circles*, 3 min; *filled triangles*, 10 min. The constant test light (zero intenstiy) gives about 90 per cent maximal b-waves in dark-adapted eye.

From the results of the experiments just described curves have been plotted which illustrate the later time course of recovery of the b-wave (beyond 30 sec). The time necessary for the b-wave (after the series of light-adaptations already mentioned) to increase from 60 to 90 per cent of its final amplitude, has been measured,

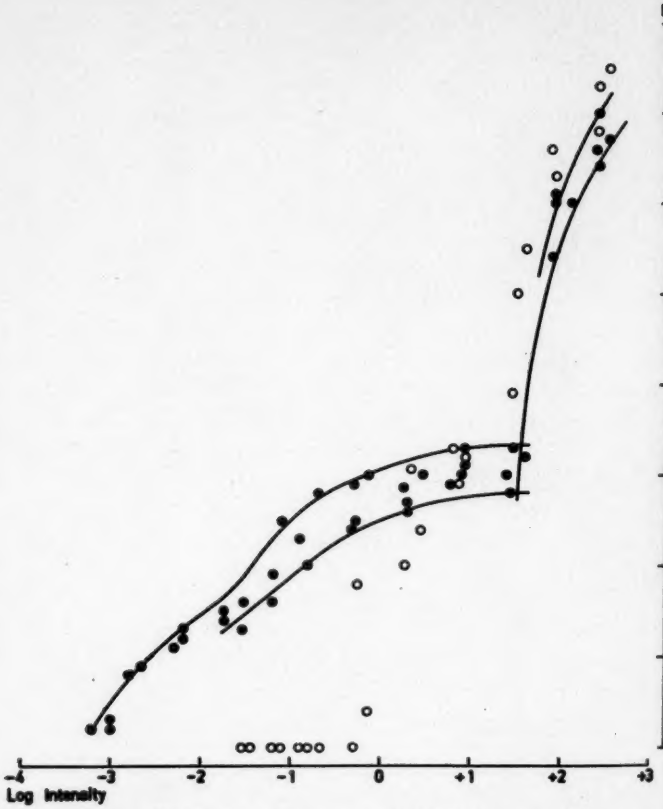

Fig. 12. Plot of flicker fusion frequency of rabbit's electroretinogram against logarithm of stimulus intensity. *Filled circles:* measurements in four dark-adapted pigmented animals. *Open circles:* (three cases) measurements after light-adaptation to 8000 lux for 15 min.

and in Fig. 11 these times are plotted against log. intensity as abscissa. The filled triangles refer to 10 min light-adaptation, the filled circles to 3 min light-adaptation. These curves also show up inflection between log. relative intensity + 1 and + 2 (*cf.* Fig. 10). The position of this inflection means that the critical intensity, at which the slow recovery sets in, is that intensity of light-adaptation

which produces maximal scotopic activation in terms of the b-wave. Intensities, about one hundred times higher, are required before the slow recovery is established.

To interpret these results we require information about the electroretinographic cone threshold of the rabbit. Therefore, in four pigmented rabbits, dark-adapted over four hours, the flicker fusion frequency was measured, and in Fig. 12, is plotted against the same scale of intensities used above.

Filled circles are measurements made in dark-adapted eyes. The fusion frequency rises along a curve consisting of two branches, break being at around 30 flashes per sec (*cf.* DODT and ENROTH, 1953; DODT and WIRTH, 1953). The upper branch approaches maximal values of about 65—70 per sec at illumination as high as 8000 lux. The break occurs between + 1 and + 2 log. units on the scale, *i.e.* at an intensity which corresponds to that at which dark-adaptation shifts from an early fast recovery to a late slow one.

It can be concluded from these results that the recovery of the scotopic b-wave is very fast below intensities which give maximal rod activation (as indicated by maximal b-wave amplitude in the fully dark-adapted eye). Above this intensity, which nearly coincides with the rabbit's electroretinographic cone threshold the slow recovery appears but is established only at about hundred times higher intensities. These also produce about maximal cone activation in terms of flicker fusion.

Effect of duration of weak light-adaptation on the recovery in the dark of the scotopic b-wave

Inspection of Fig. 10 suggests that not intensity alone but also duration of light-adaptation has an effect on the recovery of the b-wave in the dark, even at an illumination as low as log. relative intensity — 2. This was investigated in the experiments which will be described below. In Fig. 10 one can merely see a difference in the level of the curves referring to 3 and 10 min light-adaptation respectively (filled circles and triangles).

The results of Fig. 13 are from experiments on two pigmented rabbits previously dark-adapted for over four hours. The curves

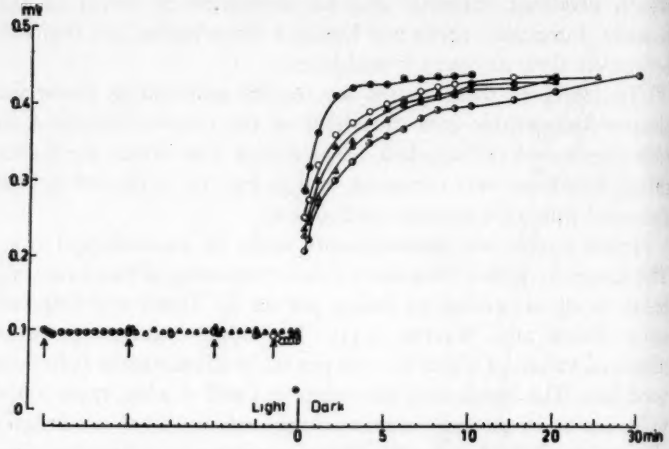

Fig. 13. Measurements of size of b-wave (averages from two experiments) evoked by constant test light during and after light-adaptation to white light of low intensity (log. relative intensity —1). The symbols used refer to the duration of light-adaptation: *open circles*, 3 min; *filled triangles*, 10 min; *open triangles*, 20 min; *half filled circles*, 30 min. *Filled circles* are measurements during and after 10 sec light-adaptation to ten times stronger light. Note the double time scale between zero and ten min in the dark. The beginning of respective light-adaptations is marked with arrows. First measurement during light-adaptation is at 15 sec (5 sec, filled circles). First measurement in the dark is 15 sec after the end of light-adaptation.

illustrated are averages of two measurements, except those marked by open and filled circles, which are averages of four.

Fig. 13 clearly shows that if the eye is light-adapted to a constant low illumination of about log. relative intensity — 1 (*i.e.* 10 times weaker than the test light) for durations of 3 min (open circles), 10 min (filled triangles). 20 min (open triangles) and 30 min (half filled circles), the time courses of recovery in the dark of the b-wave are the slower, the longer the duration of the preceding illumination. An increase of duration of light-adaption from 20 to 30 min still influences the time course of recovery (this result was found in three experiments). With this weak adapting light the reduction of the b-wave takes place so

rapidly that after the first measurement made at 15 sec there are no further changes in the size of the test response.

A check on the state of the eye was obtained by using light-adaptatation of 3 min duration in the beginning and the end of the experiment. In both cases this light-adaptation caused the same reduction of the b-wave and also the same curve for recovery in the dark. As one further check on the stability of the conditions, in both experiments light-adaptation to a ten times higher intensity of only 10 sec duration (filled circles) was carried out in the beginning and at the end of the experiment. The curve thus obtained shows the fastest rise of those illustrated in Fig. 13, in spite of the fact that it starts from a much lower level of b-wave amplitude than the others.

It is evident from Fig. 13 that even within the scotopic range of illuminations, both the duration and the intensity of the preceding light-adaptation influence the recovery of the b-wave. These results have interesting correlations in perception (SCHOUTEN, 1937; SCHOUTEN and ORNSTEIN, 1939). According to these authors the reduction of apparent brightness of an object obtained on exposure to another light source in the neighbourhood, takes place within about 0.1 sec and reaches a steady level which then is maintained. This process, called α-adaptation, is reminiscent of the rapid reduction of the b-wave at the onset of light-adaptation in Fig. 13. Similarly the β-adaptation of these authors can be related to the successively slower recovery of the b-wave from the constant level but after longer durations of illumination.

Recovery in the dark of scotopic b-wave after light-adaptation with scotopically equivalent coloured lights

In one series of experiments Balzer interference filters were used for light-adaptation at log. relative intensity — 1.0 and in another series Schott filters BG25 and OG4 at log. relative intensities + 1.2 and + 1.6 (for transmission curves of these filters see Fig. 1). Only pigmented rabbits were examined. The animals were dark-adapted over four hours before beginning the measure-

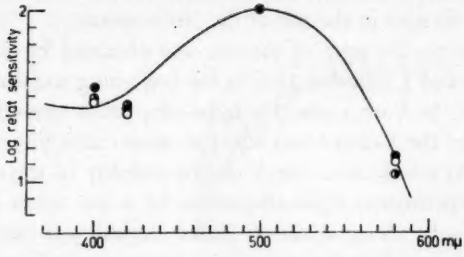

Fig. 14. Spectral sensitivity determined by Baltzer interference filters λ max. 400, 420, 498 and 580 mμ in three dark-adapted pigmented rabbits. Equal quantum intensity spectrum. *Solid line* is Wald's (1949) absorption spectrum of frog's rhodopsin. The sensitivities are calculated from measurements of equal b-waves (50 per cent maximal) which also have provided the basis for »scotopically equivalent lights» used to light-adaptation in experiments on the same animals (Fig. 15).

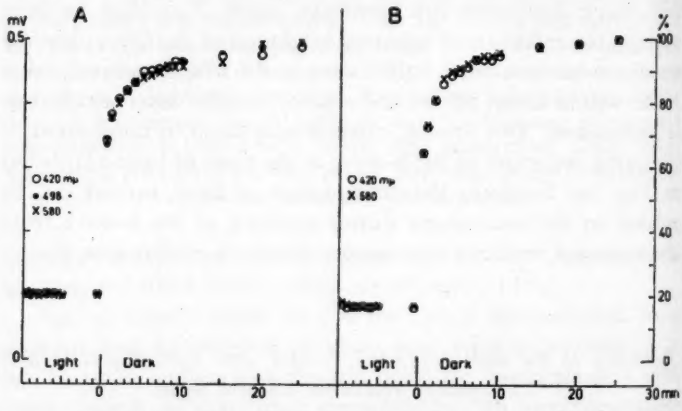

Fig. 15. Graphs illustrating reduction of the size of b-wave evoked by constant test light and recovery in the dark after light-adaptation for 10 min to scotopically equivalent coloured lights (Balzer interference filters). Intensity of light-adaptation (log. relative intensity —1) clearly below rabbits electroretinographic cone threshold. *Ordinate:* Fig. A, millivolts; Fig. B, per cent of maximal b-wave in the end of dark-adaptation. *Abscissa:* time in minutes from the end of light-adaptation.

ments. The ERG has been elicited with 0.2 sec flashes of the green test light.

Because of individual variations in the spectral sensitivity of rabbit's retina, the scotopical equivalents of lights used for light-adaptation have been measured for each eye separately using 50 per cent maximal b-waves as an index. Use of equal b-waves, instead of equal visual purple absorption, in comparing effects of lights of different wave-lengths also made corrections for absorption and possible fluorescence in the eye media unnecessary. It can be seen in Figs. 2 and 14 that the electroretinographically measured relative sensitivities deviate from the visual purple absorption curve at 400 and 420 mμ. Further deviations from this curve at 460—470 mμ were found often but not always.

In three rabbits the energies giving equal b-waves were measured using Balzer interference filters (λ max. 400. 420, 498 and 580 mμ). In Fig. 14 reciprocals of these energies are plotted as log. relative quantum sensitivity. In one rabbit (filled circles) light of wavelength 420, 498 and 580 mμ were used for light-adaptation at the highest possible intensity (maximal energy transmitted by the filter λ max. 420 mμ). Fig. 15A shows that these scotopically equivalent spectral lights cause equal reductions of the test b-wave when used to light-adapt the eye (length of adaptation 10 min, b-wave tested after 30 sec and then every minute up to 5 min). Also the recovery curve (followed for 25 min) is the same after anyone of the light-adaptations, *i.e.*, the wave-length used makes no difference. Fig. 15B illustrates similar results found in the other two cases (open and half filled circles in Fig. 14). Both these eyes were light-adapted with light of wave-length 420 and 580 mμ only, in one case the first and third light-adaptation was with light of wave-length 420 mμ and in the other with 580 mμ respectively. Consequently the symbols (open circles and crosses in Fig. 15B) represent averages of three measurements.

In all three cases the coloured lights reduced the test b-wave to the level of about 20 per cent of maximal b-wave amplitude as found in the respective experiments. In the individual experiments the differences in the magnitude of the reduction caused by different colours fell within limits of 2—3 per cent.

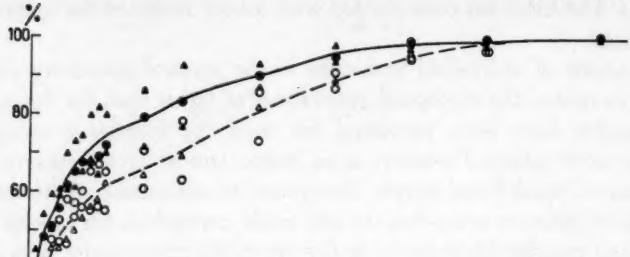

Fig. 16. Measurements of size of b-wave in two pigmented rabbits (*circles and triangles*) during dark-adaptation after light-adaptation to scotopically equivalent blue and orange lights (Schott Jena filters BG25 and OG4). Light-adaptation for 30 min to log. relative intensity + 1.2. *Ordinate:* per cent of maximal b-wave in the end of dark-adaptation. *Abscissa:* time in minutes from the end of light-adaptation. *Solid and broken lines* are drawn through averages of measurements after blue (filled symbols) and orange (open symbols) light-adaptation respectively.

In four rabbits the Balzer interference filters were replaced by Schott filters BG25 and OG4 in order to have more intense light-adaptation. For measurement of equal scotopic b-waves the intensity of these lights was reduced some hundred times by diminishing the aperture of the shutter. For broad band filters this procedure was found to be more accurate than that of using neutral filters which deviate somewhat from perfect neutrality (it is impossible to calculate the required wedge setting during the experiment).

The results shown in Fig. 16 are from experiments on two rabbits (circles and triangles). In these experiments the duration of light-adaptation was 30 min at log. relative intensity + 1.2. Similarly Fig. 17 illustrates experiments on two animals (circles and triangles) light-adapted for 10 min at log. relative intensity

+ 1.6. In both Figs. 16 and 17 filled symbols represent measurements after light-adaptation with blue, and the open symbols with orange light respectively. In every experiment three light- and dark-adaptations were measured. An equal number of experiments was begun with light-adaptation to blue and orange light respectively and in every experiment the first and third light-adaptation was performed with the same colour filter. All measurements have been given in per cent of b-wave amplitude in the end of dark-adaptation.

It is evident from Fig. 16 that even at log. relative intensity + 1.2 the recovery is slower after light-adaptation to orange than to scotopically equivalent blue light. At log. relative intensity + 1.6 (Fig. 17) the difference in the effect of the orange and blue light is slightly greater, despite the shorter duration (10 min) of light-adaptation. Fig. 18 shows some records from one of these experiments. The b-waves are clearly larger after blue than after orange light-adaptation. For example at 15 min in the dark the b-wave is 70 per cent maximal as compared with only 54 per cent in the controls.

In Figs. 16 and 17 the solid and broken lines are drawn through averages of measurements after light-adaptation with blue and orange light respectively. An interesting comparison can be made between the curves of Fig. 17 and the curves obtained after different intensities of light-adaptation with white light of 10 min duration (Figs. 10 and 11). Fig. 17 shows that the average recovery at 30 sec in the dark (starting points of the curves) is 18 per cent of maximum after orange and 26 per cent after blue light-adaptation. In Fig. 10 it can be seen that the difference between the 18 and 26 per cent recovery (in 30 sec) corresponds to a difference of 0.2 to 0.3 log. units in the intensity of white adapting light. The same result is obtained if the recovery times from 60 to 90 per cent level (in Fig. 17, 37 min solid line and 44 min broken line) are related to the curves illustrated in Fig. 11. A third comparison has been made between the curves of Fig. 17 and the average curves of Figs. 8 and 9. In Fig. 19 the percentage recovery 10 min after light-adaptation is replotted. The symbols refer to the intensities of white adapting lights and are the same as in Fig. 8.

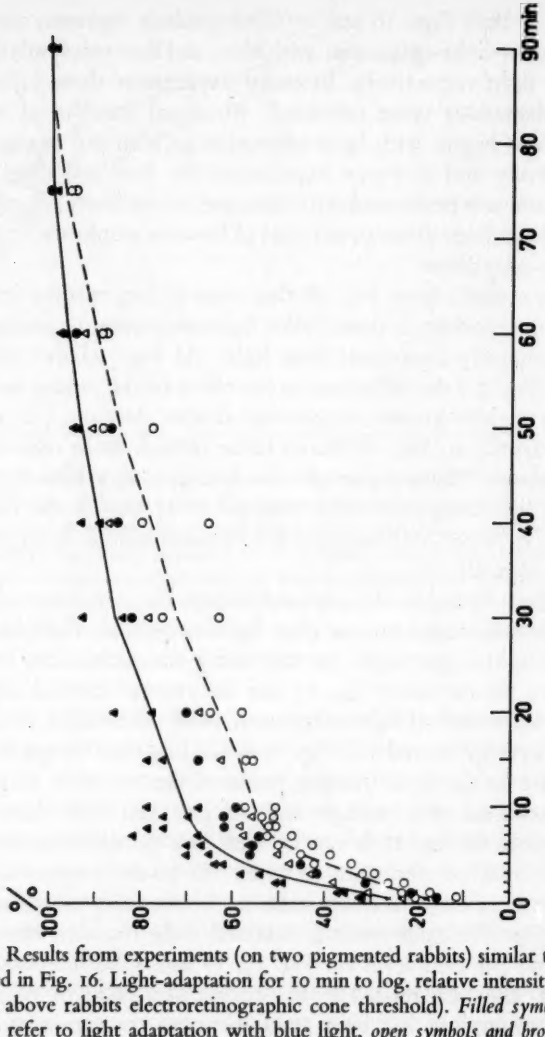

Fig. 17. Results from experiments (on two pigmented rabbits) similar to those illustrated in Fig. 16. Light-adaptation for 10 min to log. relative intensity + 1.6 (slightly above rabbits electroretinographic cone threshold). *Filled symbols and solid line* refer to light adaptation with blue light, *open symbols and broken line* to orange light respectively.

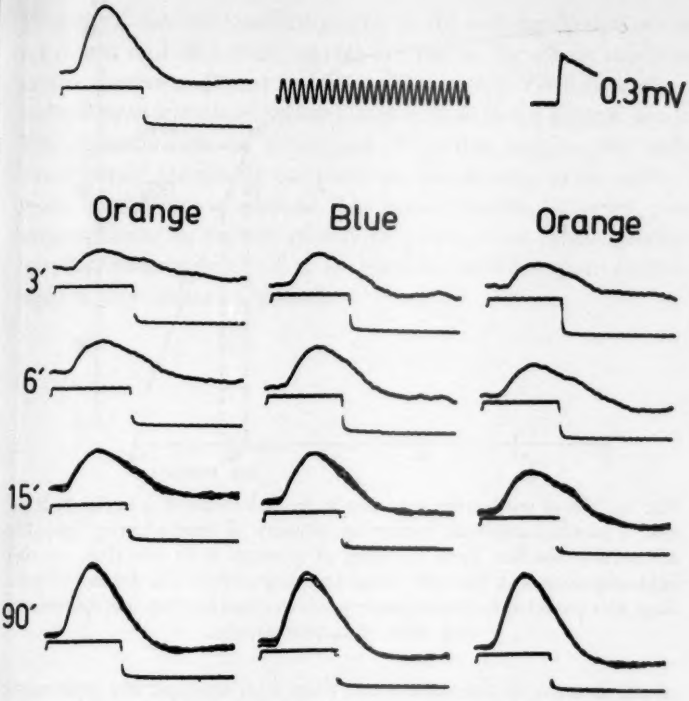

Fig. 18. Records illustrating the recovery in the dark of the test b-wave after light-adaptation to orange light (first and third column) and to scotopically equivalent blue light (second column). Intensity of light-adaptation slightly above rabbits electroretinographic cone threshold. Single b-waves at 3 and 6 min in the dark. Three b-waves superimposed at 15 and 90 min. The record in the upper left corner is a control before the first light-adaptation. Time-mark 50 c/s. Calibration 0,3 millivolts.

The horizontal broken lines show the levels of recovery of the test b-wave at 10 min (Fig. 17) after light-adaptation with blue and orange light respectively. The distance between these levels projected onto the abscissa, again corresponds to about 0.25 log. unit difference in the effect of light-adaptation.

The results show that at intensities above the rabbit's electro-retinographic cone threshold orange light slows down the recovery

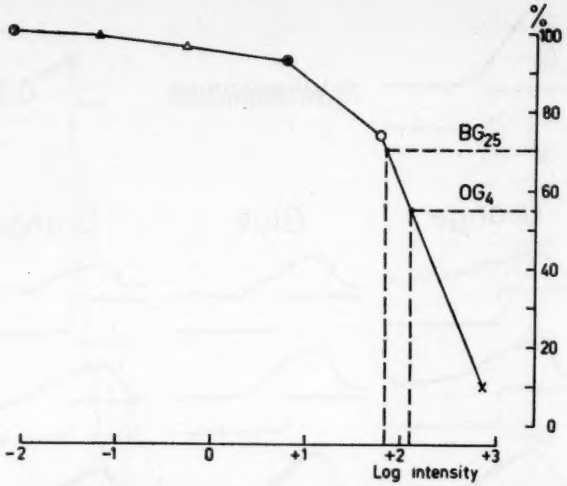

Fig. 19. Size of test b-wave at 10 min in the dark (symbols as in Fig. 8) in per cent of maximal amplitude against log. intensity of white adapting light. The *horizontal broken lines* show the levels of recovery at 10 min (Fig. 17) after light-adaptation with blue and orange light respectively. The distance between these lines projected to abscissa, corresponds to about 0.25 log. unit difference in the effect of light-adaptation.

of the b-wave in the dark more than does scotopically equivalent blue light. This effect was seen in four animals and it lasted about one hour. As found in the previous sections, slow recovery required light-adaptation bright enough to activate cones, as shown by the flicker ERG. Orange light is likely to stimulate cones more effectively than scotopically equivalent blue light.

The difference in the effect of the orange and blue lights, found to be the order of 0.25 log. units, is far too small to correspond to a full Purkinje shift. When measured subjectively by the author (using the same optical arrangement as for the rabbit), the full Purkinje shift changed the relative sensitivity for the orange and blue lights (OG4 and BG25) by about 2.0 log. units. Consequently a difference of 0.25 log. units would correspond to a shift of spectral sensitivity by something of the order of 10 mμ only. A definite 'Purkinje shift' has been demonstrated

by using flicker electroretinography in the rod dominated eye of cat (DODT and WALTHER, 1958*d*) but not in the albino rabbit in similar conditions (DODT and WALTHER, 1958*e*). Yet these authors had coloured stimuli of higher intensity than in the present work. The measurements of DODT and WALTHER suggest that only visual purple containing receptors are functioning in the rabbit's retina in photopic conditions. The present results, however, give some evidence for activity of 'red' or 'green' cones. Their isolation by electroretinography, if at all possible, would require exceedingly intense coloured stimuli.

DISCUSSION

It has been found in this work that the slow dark-adaptation of rods after intense light-adaptation requires adapting stimuli of the strength necessary for cone flicker fusion. It is assumed that under these circumstances retinal function is re-adjusted in such a fashion as to bring into prominence another system which briefly may be referred to as 'cones'. Further evidence for this view comes from the experiments with coloured light adaptations described in the previous section. When scotopically equivalent orange and blue lights were used for light-adaptation at intensities above the electroretinographic cone threshold, the orange light was found to slow down recovery more than the blue light though not if the lights were below cone threshold. This result suggests that the effect of the intensity of intense coloured lights on the time course of dark-adaptation of rods is determined by their photopic intensities rather than by their scotopic intensities. A small 'red shift' of spectral sensitivity after light-adaptation was deduced from the recovery curves with blue and orange light respectively.

The results of ELENIUS and HECK (1957, 1958) provide further support for the theory above. In two totally colour blind human beings the scotopic b-wave was found to reappear immediately after adaptation with an intense white light (1000 lux, 10 min), while in normal controls there was no b-wave for several minutes. In these totally colour blind subjects the maximal flicker fusion frequency did not exceed 25—30 flashes per sec, and above 100 lux the electrical response disappeared.

An inhibitory effect of active cones on rod function has been earlier assumed by GRANIT and WREDE (1937) and GRANIT and TANSLEY (1948). Also a reversal of this effect, dark-adapted rods suppressing the cones, has been used by GRANIT and RIDDELL

(1934) and GRANIT (1935) to explain differences of the electrical responses of mixed and pure cone retinae in light- and dark-adaptation. GRANIT (1938) suggested at the time that the momentarily more active receptor system, favoured by the prevalent conditions of illumination, occupies the common pathways so that the other, though photochemically active, is excluded. POLYAK (1941) attributes inhibitory properties to the horizontal cells of the retina. He describes these cells as making connections between the cone pedicles and the vitreal ends of both rods and cones. GRANIT and TANSLEY (1948) suggested that the inhibitory process occurs in both amacrine and horizontal cells.

In the present work it has been shown that in the rabbit eye rod function can be suppressed for several hours after bright light-adaptation of long duration. This means, that the systemic shift assumed to slow down recovery of the b-wave must remain active for a long time after cessation of illumination. Indeed, the release of rod function can be so slow that maximal b-waves are found only after four hours in the dark. The results therefore suggest that the main factor which determines the time course of dark-adaptation of rods after intense light-adaptation is a systemic reorganization in the retina. In the favour of this idea are the facts that after an intense enough light-adaptation the maximal concentration of regenerated visual purple is found to correspond to only about 20 per cent recovery of the b-wave, and that maximal recovery time of b-wave can be up to two hours in excess of the time for maximal regeneration of visual purple. At this moment of full recovery, the eye was in excellent condition, as shown by the experiment of Fig. 7.

Another interesting result of this work is the slower dark-adaptation after longer durations of light-adaptation of low intensity (below electroretinographic cone threshold). SCHOUTEN (1937) pointed out the similarity between subjective α-adaptation and the negative inhibitory component P·III of the ERG-response (GRANIT, 1933). In the present experiments the very rapid reduction of the b-wave to a constant potential level, as caused by weak illumination, is similar to SCHOUTEN's α-adaptation. Similarly the slower recovery of the b-wave after longer durations of light-

adaptation of constant intensity resembles his β-adaptation. From the earlier work of GRANIT, HOLMBERG and ZEWI (1938) it is known that in the frog, weak illumination causing considerable reductions of b-wave is not associated with any measurable breakdown of visual purple. RUSHTON (1953), by measurements *in vivo*, has found that only light of an intensity of 10^6 times the human absolute threshold bleaches visual purple in the retina of the albino rabbit. In the present experiments the corresponding intensity (measured by the author to be log. relative intensity — 1) has been found to reduce the b-wave of pigmented rabbits to about 20 per cent of the maximum. Thus, in the rabbit α-adaptation has been demonstrated at intensities of light-adaptation which evidently do not alter the visual purple concentration (in the pigmented eye brighter light must be used to obtain the same bleaching effect as in the albino retina). Also, the great rapidity of α-adaptation is against a photochemical explanation (*cf* SCHOUTEN, 1937).

According to RUSHTON *et al.* (1955), in the rabbit visual purple bleaching and regeneration reach a steady level after about 10 min of constant illumination. However, after weak light-adaptation which does not abolish the electrical response to the test light, the recovery of the b-wave is found to be slower if the duration of light-adaptation is lengthened from 10 to 20 and 30 min. This suggests that β-adaptation is not related to alterations of visual purple concentration, in spite of the possibility that in light-adaptation of the strength used for demonstrating the process the equilibrium density does change. If the amount of breakdown of visual purple were assumed to determine the time course of recovery of the b-wave in β-adaptation, it would be difficult to explain that recovery is faster from a definitely lower potential after 10 sec exposure than from a larger potential obtained after adaptation to weaker light of longer duration (as illustrated in Fig. 13).

RUSHTON (1957) has found that *in vivo* the regeneration of visual purple takes the same course after scotopically equivalent yellow and blue illumination. Apparently in the living human eye irradiation of retinene is not a limiting factor for visual purple regeneration, as it is in solution (HUBBARD and WALD, 1952). For

the present work this is important, as it excludes the possibility that the faster recovery of the b-wave after blue than orange illumination could be due to faster visual purple regeneration in one case than in the other. The differences described in Figs. 16 and 17 cannot therefore be based on differences in the regeneration of visual purple. Since visual purple regenerates at a fixed rate *in vivo* (*cf.* LEWIS, 1957), the explanations of differences in rate of dark-adaptation under various conditions (see Introduction), proposed by HECHT, HAIG and CHASE (1937) and WALD and CLARK (1937) cannot be valid. Clearly, however, the present results provide alternative explanations of these well known facts.

GRANIT, THERMAN and WREDE (1938) demonstrated in the frog that blue and violet monochromatic light-adaptation reduced the b-wave (evoked with a constant green test light) less than scotopically equivalent lights of long wave-lengths and that the recovery also was faster after light-adaptation to short wave-lengths. These results, confirmed with the rabbit's eye with lights above 'cone threshold' (Figs. 16 and 17), can also be explained by supposing that rod function is suppressed by activated cones. The electroretinographic cone threshold of the frog is at a considerably lower intensity than that of the rabbit, so GRANIT *et al.* (1938) could use weaker lights than in present work. The rabbit's retina is dominated by rods (SCHULTZE, 1866; KRAUSE, 1881) whereas there is about the same number of rods and cones in the frog retina. The energy of the Hilger-Tutton monochromator used by GRANIT, THERMAN and WREDE (1938) (used also in the present work) was high enough for demonstration of a Purkinje shift in the frog (GRANIT and WREDE, 1937). This explains why their results could be repeated in the rabbit only at a much higher intensity of coloured light-adaptations.

As early as in 1924, MÜLLER explained the variations in rate of dark-adaptation, discussed in the Introduction, by a suppressing effect of activated cones on the rods. The idea is similar to the one proposed in this work but MÜLLER thought in terms of visual purple regeneration which clearly does not vary (*cf.* above) very much. He called the inhibition of regeneration 'rhodogenetische Hemmung' and drew his example from differences concerning

photophobia, intensity discrimination and dark-adaptation in the totally colour blind and the peripheral retina of normal man. In the present work the emphasis is laid on systemic adjustment so that, when a mixed retina has been adjusted for performance at cone level by sufficiently intense adapting lights, the rod system is suppressed.

SUMMARY

1. The recovery in the dark of rabbit's electroretinogram has been studied. The intensity of preceding light-adaptation has been varied over a range exceeding 6 log. units. The duration of light-adaptation has been varied from 10 sec to 30 min. The test electroretinogram has been evoked with a monochromatic green light which in the fully dark-adapted eye gave about 90 per cent maximal response. This test light has been constantly used throughout this work.

2. After intense light-adaptation of long duration (8000 lux, 30 min) there is a delay of about 10 min before the b-wave reappears and further recovery is very slow. At 90 min in the dark the test b-wave has recovered to only some 20 per cent of maximum. Yet in this time the total quantity of visual purple is restored (*in vivo*, measurements of RUSHTON *et al.* (1955)). Maximal b-waves, however, are found only after at least four hours in the dark.

3. The recovery of b-wave has been found to be very fast after light-adaptation at strengths below the level necessary for maximal rod activation, as judged by the size of the b-wave in dark-adaptated eye.

4. When the intensity of light-adaptation is increased to a level that also activates cones, as judged by the electroretinographic flicker fusion frequency, the slow recovery first makes its appearance. Further increase causes slower recovery of the b-wave.

5. At an intensity above the electroretinographic cone threshold light-adaptation with orange light slows down the recovery of the b-wave more than scotopically equivalent blue light. This is not the case at an intensity of light-adaptation clearly below the cone threshold. The effect of intense coloured lights on the course of recovery of b-wave is assumed to be determined by their relative photopic intensities. From the difference in the rates of

recovery after orange and blue light-adaptation it has been calculated that the photopic spectral sensitivity maximum is displaced 10 mμ towards the red.

6. Light-adaptation with weak light (defined as light which does not abolish the test b-wave) causes an immediate reduction of the b-wave to a constant level of potential which then is maintained. This fast reduction in size of b-wave is similar to human sensory α-adaptation. The rate of recovery in the dark following such weak light-adaptations is slower, the longer the duration of light-adaptation. This resembles the sensory β-adaptation. The relation of α- and β-adaptation to visual purple regeneration is discussed.

7. Light-adaptation with more intense light (above the electroretinographic cone threshold) is assumed to readjust retinal function so as to bring another system, 'the cones', to dominance while 'the rods' as a system are suppressed. This suppression may last for several hours after the end of light-adaptation. It is suggested that the release of rod function from this suppression rather than visual purple regeneration, determines the course of the delayed slow dark-adaptation of the rod system as measured by the b-wave.

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